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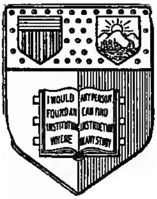
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KIUTSI'S

ISOLATION OF PROTECTIVE FERMENTS

AND

PROGRESS OF FILTRATION PROCESS.

Dr. M. Kiutsi.

Director of women dicease Department
of the City-hospital at Hakodate.

Octover 1, 1914.

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ISOLATION OF PROTECTIVE FERMENTS,

AND

PROGRESS OF FILTRATION PROCESS.

Great is the power of the truth. Wake my friends, and be sagacious and sure. The truth is about to embrace us. The hunter can certainly get the game if he uses the right method of hunting. The fisher can easily catch the fish unless his method of fishing is wrong. Stand up, my friends, and catch the truth and be with it. The truth never escape us if we use the right method to catch the truth in our investigation.

Since I built the foundation for urin-diagnosis, the world has already recieved it with great enthusiasm, and I consider it my honor and pleasure to see that the public pay such an interest to my new method since my publication four months ago.

Now the fields are turning gray telling us the coming of another season, and I marched into the second stage of my investigation perfecting my filtration process. It became decidedly distinct, and it is not at all difficult with the improved mothod to diagnose the pregnance in but two hours after the test began. Before describing it, let me tell what my second stage of investigation is.

What is the second stage?

It is the *isolation of protective ferments—schütsfermente.*

Though my filtration method proves the presence of the ferment indirectly, the direct confirmation of its presence has not yet been reached. But, as there was an indirect confirmation of its presence, its presence ought to be proved directly by some means, and I went on finding means to prove its presence directly, realizing that it is my duty to do so. Without any one to consult with, I battled by the lead of certain belief in my mind for two years, some time loosing and some time winning. At last I succeeded in isolating it.

Now allow me to tell the passage of its isolation.

Since the first part of August this year I wanted *to test the change in action of protective ferment under various circumstances and studied under different physical and chemical conditions.*

Firstly I wanted to know the action of sun light. I exposed the pregnant's urin to sun light by a glass window for one month, and I tested so treated urin by my filtration method. But no change in its action was noticed.

Secondly I tested out the action of X–ray on the protective ferment of pregnant's. In this test, the X–ray of 5 mili-amperes was exposed to pregnant's urin at a forcus of 20 cm. for 30 minutes. The result showed that such an exposure not at all diminished the action of ferment.

Thirdly, to find the influence of strong acid solution toward the ferment I used phosphorus wolfram acid. By means of this acid the amino acids and a part of protein in the urin were precipitated. The supernatant liquid was taken and neutralized with caustic magnesia. After these treatments the urin was dialyzed by my improved dialyzing method, and it was found to be possitive.

For the fourth place, to see the influence of alkalis upon the protective ferment a pregnant's urin was made alkaline with a strong solution of sodium hydroxide. After this, the urin was filtered by my filtration process and the reaction was again possitive.

For the fifth place, to find if symbiosis of various bacteria would not do any harm to the protective ferment I intendedly let the urin to ferment. After this the urin was first filtered through a bacterial filter and next by my filtration method. The result was still possitive to the protective ferment.

For the sixth place, to determine the influence of temperature upon the action of protective ferment I first put a pregnant's urin in ice for 24 hours, and it was tested with possitive result. Therefore, I proceeded to test with following temperatures.

Temperature	Time exposed	Reactions
40° C.	30 min.	Possitive
50° ,,	30 ,,	,,
60° ,,	30 ,,	,,
70° ,,	30 ,,	,,
80° ,,	30 ,,	,,
90° ,,	30 ,,	,,
100° ,,	30 ,,	,,

From the above data I found that the action of ferment became weak upon exposure to 100° C. for 30 minutes. Therefore, I immediately took up the following test with higher temperature.

Temperatures	Time Exposed	Reactions
110° C.	30 min.	Very weak
120° C.	30 ,,	Negative

From the above test it can be seen that *the action of what I named protective ferment is weakened at temperature avobe 100° C. and entirely destroyed at 120° C.*

As I finished such tests as described thus far, I thought out of the following hypothesis.

That if a pregnant's urin be dried up under 90° C., my so-called protective ferment will be alive in the residue.

If so, I must be able to grasp out and expose the protective ferment before my eyes. Then I stormed toward this object with a great interest.

Though before I could reach to the goal of my object I met many obstacles, finally I was able to get the much wanted protective ferment into a bottle.

Hereon let me tell about the method of its isolation as performed by me.

I first took a healthy woman's urin who had been pregnant for 5 months. It was repeatedly filtered through animal char-coal or blood-char-coal to remove urin pigment, protein and peptone whose removal being tested by my Bieuret reaction method. The filtrate was taken into Bechel glass and placed on double water bath to dry it at a temperature lower than 90° C. When crystals appeared on the surface they were taken out with filter paper and filtrate was again vaporized. When this was continued, on the bottom and periphery of the Bechel-glass a very thin mica-like substance appeared. This substance was collected with a spatula. This collected substance was the desired protective ferment.

I call the above substance for the sake of simplicity "*pregnant ferment.*"

I am at the present making a progress in preparation of purer pregnant ferment and believe that I can get pure ferment some time. But, just at this moment my pregnant ferment is prepared in the manner already described. At any rate I must say a word in regard to large white crystals and needlelike crystal which adhere to the bottom of Bechel glass. They have nothing to do with the ferment and their detailed description will be published later.

Though the pregnant ferment was thus isolated, whether or not it is the protective ferment which I was studying thus far, must be decided with the ultimate experiment.

I shall now describe the experiment.

To test the action of pregnant ferment on Kiutsi's pregnance-diagnosing powder "Ninserin" which has been prepared from a certain element of afterbirth by my special method, I took 0.05 gram of Ninserin into a test tube and 2 ear-pickfuls of the pregnant ferment and 10 cc. of distilled water were added. After a little shaking the content was left for two hours and filtered it into another test tube with filter paper. To the filtrate 0.2 cc. of 1 % ninhydrin solution was added and boiled for one minute. It was again left at a room temperature, and within 15 minutes a very beautiful purple color appeared which shows the possitive reaction. (As to the control test see later paragraph.)

From the above it was known that the pregnant ferment resolves Ninserin into peptone and amino acides, i. e., that the isolated ferment is the same with the ferment hitherto dealed with in my filtration process.

Encouraged by the above, I further proceeded to make various other tests and *cauld know the following properties.*

1st. The pregnant ferment can readily be dissolved in water.

2nd. The water solution of pregnant ferment is neutral.

3rd. The water solution of pregnant ferment is negative to Bieuret and ninhydrin reactions.

4th. The water solution of pregnant ferment markedly looses its activity when heated to 100° C. for 30 minutes and the power of resolving Kiutsi's pregnance-diagnosing powder is greatly diminished. When it is heated to 120° C. to 130° C., its activity is totally destroyed.

5th. If the water solution of pregnant ferment is treated with alcohol, the ferment gradually precipitates and in a few hours it is settled on the bottom of test tube in a mass.

6th. When the pregnant ferment is exposed to the air, though once dried, it becomes dampy by absolving moisture from the air.

The above properties are what I have already ascertained up to date and the more minute properties will be determined later.

As the facts are so clear as hitherto shown, I shall leave the further discussion on this point out, and step directly into the practical side of this question. For this purpose, I shall describe the progress of Kiutsi's filtration process.

Materials Necessary For The Test.

1. Kiutsi's pregnance-diagnosing powder "*Ninserin*"
2. Animal char-coal
3. Filter paper.
4. Funnel.
5. Bechel glass.
   This is the receptacle for the filtered urin.
6. Big test tube.
   This is convinient for boiling.
7. Test tube holder.
   This is convinient for boiling.
8. 1 % ninhydrin solution.
   This is to be kept in a small dark bottle.
9. 0.2 cc. capillary pipette.
10. 10.0 cc. measuring cylinder.
11. Distilled water.
12. Bunsen burner or alcohol lamp.

Only the above twelve are all it needs.

The procedure of the test with above materials is given subsequently.

Performance of Test.

At the first place a pregnant's urin is filtered to remove urin-pigment and protein, and the filtrate is put into Bechel glass. Then it is dried at a temperature under 90° C. Two ear-pickfuls of the micalike residue of the urin are taken into a test tube, and to it 0.05 gram of Ninserin and 10 cc. of distilled water are added. After shaking a little, the content is left at a room temperature for two hours. Then it is filtered through filter paper into another test tube. To the filtrate 0.2 cc. of ninhydrin solution is added. This is slightly shaken and the test tube is held by a test tube holder and placed over a flame, boiling the content for about one minute. Leaving it at a room temperature for 15 minutes a beautiful purple color appears. It shows the possitive reaction.

Experimental Steps.

The experimental steps are divided into three, namely the preliminary, the main and the control.

The Preliminary Experiment.

On this experiment, the change of Kiutsi's pregnance-diagnosing powder "Ninserin" in distilled water was determined.

No.	Ninserin	Distilled water	Reaction.
1	0.005 grm.	10.0 cc.	Negative
2	0.01 "	10.0 "	"
3	0.02 "	10.0 "	"
4	0.03 "	10.0 "	"
5	0.04 "	10.0 "	"

6	0.05 grm.	10.0 cc.	Negative
7	0.10 „	10.0 „	„

From the above, it can be seen that Ninserin does not produce any substance that reacts toward ninhydrin when it is left with distilled water.

The Main Experiment.

On this experiment it was endeavored to see if Ninserin be resolved by pragnant's urin residue dissolved in distilled water.

No.	Ninserin	Pregnant's Urin Residue	Reactions.
1	0.05 gram	2 ear-pickfuls	Positve
2	0.05 „	————	Negative
3	——	2 ear-pickfuls	Negative

The avove data show that Ninserin is resolved by pregnant's urin residue, or what I said pregnant ferment, into peptone and amino acids.

The Control Experiment.

On this experiment whether or not the Ninserin be resolved by urin residues other than pregnant's was tested out.

No.	Ninserin	Urin Residues 2 ear-pickfuls each	Reactions
1	0.05 gram	Muscle tumor	Negative
2	0.05 „	Consumption	„
3	0.05 „	Man's	„

The above data show that the Ninserin can not be resolved by urin residues other than the pregnant's.

Taking all the experiments into consideration, it is clear that the pregnant's urin residue contains pregnant ferment, and more-over its activity toward the Ninserin is very marked with ninhydrin, exposing the reaction before our eyes within only two hours.

It takes, however, experience to distinguish the protective ferment in the residues on the bottom of Bechel glass. It is very likely to collect amino acids such as leucin and tyrosin, and it is what I fear more than any thing else in this test.

Hereon I got an idea and repeatedly filtered the filtrate which became negative to Bieuret reaction by filtering urin through animal char-coal. By doing this it was found that the ninhydrin reaction became fainter each filtering. Encouraged by this phenamenon, I continued with this process of filtration time after time. As nearly all the urin was absorbed by animal char-coal, it was extracted with distilled water equal in amount to the original volume of urin, and it was again filtered through new animal char-coal. *After repeating this for a few times, the ninhydrin reaction became negative.*

So treated distilled water extract of urin which became negative to ninhydrin reaction was tested out as to its action toward Ninserin.

The reaction was positive!

And the reaction was very marked in two hours! .

At this point it is apparent that my filtration method may be performed in three ways.

The first is to make urin to be tested pasty with animal char-coal and left for a few hours. Next this is extracted with distilled water equal in its amount to the original volume of urin, and filtered through filter paper. This filtrate was repeatedly filtered through animal char-coal until ninhydrin reaction became negative.

The second is to double the urin in its amount with distilled water at its beginning, and the filtration through animal char-coal is to be patiently conducted until the filtrate becomes negative to ninhydrin reaction.

The third is to heat the urin to be tested to about 50° C. and then into it animal char-coal is added. Employing the first method, the water extract was made negative to ninhydrin reaction. This heating removes a part of tyrosin and makes action of animal char-coal quick.

On the above three ways, I prefer the second for adaption of the public. Therefore, I shall subsequently describe this method as standard method. But, the readers may act according to the exigency of the moment.

The Progress of Kiutsi's Filtration Process.

Dilute the urin to be tested with equal amount of distilled water and repeatedly filter through animal char-coal or blood char-coal until negative to ninhydrin reaction. Next take 10 cc. of this clarified urin into a test tube and add 0.05 gram of Ninserin and left at a room temperature. After two hours the entire liquid is filtered through filter paper into another test tube, and the filtrate is subjected to ninhydrin or Bieuret test. If the reaction is positive the person under consideration is pregnant, and otherwise she is not pregnant.

The time lost in this method of filtration can easily be made up by the time gained in the reaction.

But in case ninhydrin reaction is taken during the filtration process to test the completion of the filtration, it is adviced to take a small amount of urin instead 10 cc., regulating the amount of ninhydrin accordingly, thus saving the urin.

At this point I have a few words to add.

It is to explain why I was compelled to use Bieuret test for my old filtration method. The reason was because I feared the presence of amino acids in patient's urin and in urin of persons physiologically exited. (See Urin-diagnosis by means of dialyzing method by Kiutsi in Igaku-Chuwo Zasshi, Oct. 20, 1914.) But during the isolation of my pregnant ferment, it was found that the amino acids can be removed by repeated filtration to such a degree that the filtrate is no longer possitive to 0.2 cc. of ninhydrin solution. The use of Bieuret test is to detect the presence of peptone as the result of the action of pregnant ferment upon protein. The ninhydrin, on the other hand, acts not only on peptone but also on amino acids, thus making the reaction more marked. Now I can confidently use ninhydrin in my new method.

Now the truth of my filtration method became more apparent by my new method. The isolation of pregnant ferment has long been my ideal since I was in Europe, and this idea never parted a moment from my mind. As I was studying the problem logically, I finally succeeded in isolating it, and this certainly satisfies a part of my mind at least. At this chance I cordially extend my gratitude to my benevolent teachers and predecessors for their encouragement and pay respect to the labor of young investigators.

And I beg the public at this chance for their kindness in giving me honest advice forever.

As to the synopsis of my new filtration process, I have already described, and I am confident that the readers can well understand it. Now I want to step a little further in my investigation and try to purify the protective ferment and study various protective ferments. For, though my pregnant ferment as isolated by the method already described is no doubt my desired placenta protective ferment in its greater part, it is also possible that there may be various other protective ferments that are associated with abnormal conditions resulted from pregnance. It has been my ideal to isolate the pure placenta protective ferment from the mixed ferments of the pregnant period. On the other hand, it can not be said too hastily whether or not protective ferments other than pregnant's have the same properties with pregnant ferment toward heat, activity and other conditions. Though various protective ferments are no doubt more or less same according to my experience, they may differ in certain respects. Therefore, I wait the conclusion on this point until later study. I am making these statements in order to avoid misunderstanding of the public.

The protective ferments that I could isolate up to date are as follows:

1. Pregnant ferment.
2. Comsumptive ferment.
3. Cancer ferment.
4. Gall bladder disease ferment.
5. Appendicitis ferment.
6. Retinitis ferment.
7. Chorioiditis ferment.
8. Crystallitis ferment.

The above showed me very interesting phenomena, and I believe that there will be a chance to describe them later.

Those who understand the success of my new urin-diagnosis filtration method would ask at once the following question.

Can it be possible to improve the old method of dialysis in blood-serum diagnosis?

I have been thinking of this for a long time and found out a very interesting and profitable new method.

As to the *Kiutsi's improved dialysis* about dried placenta, I have already described in Tokyo Iji Shinshi No. 1876, June, 1914. At that time the improved method was much better than the original method in which boiled placenta was used, and by it only the pregnant's bloodserum diagnosis became practical. But I did not try to get satisfied entirely with this improved dialysis.

Why?

Unsatisfactory points in Kiutsi's improved dialysis are in the troublesomeness of cleaning dialyzing tube after its use and also in that as dried substratum sticks to the inside of the tube it is likely to become useless after a few performances. As the important point in practical application is in the cheapness of any material to be used. Therefore to satisfy my want I thought out a new method endeavoring to prolong the life of the tube. As I believe this new method is not only very economical but also very certain, I shall subsequently describe the method.

Because this new method is first discovered by myself, I shall call this method for the sake of convinience *"Kiutsi's new dialyzing process."*

Let me again tell about the course of the discovery of this new method of blood serum diagnosis.

As to the discovery of the facts that the protective ferments resolve peptone and amino acids into their last products in dialyzate has already been published.

To prove this I performed a test as follows. Into a dialyzing tube 10 cc. of blood serum and equal amount of distilled water were put in. This was left at a room temperature for 10 hours. After this the dialyzate was tested as follows:

Taking 3.0 cc. of dialyzate into another dialyzing tube, and into it 0.05 gram of Ninserin was added. This was put into a vessel containing 10 cc. of distilled water and left at a room temperature for 10 hours. The final dialyzate was all taken into a clean test tube and ninhydrin reaction was tested. The result was positive with beautiful color.

This test clearly proves that the ferment passes out to the outer liquid through dialyzing membrane.

I believe that the observations are now saficient.

The matter that concerns more is the scientific application of these observations, and now listen me to that matter.

At the moment of this observation I got the following thought:

If the protective ferment passes freely out of dialyzing tube, the result must be the same when the substratum be placed from the beginning at the ***outside*** *of the tube, i. e.,* ***in*** *the water container. As the Kiutsi's dried substratum a property of not going through filter paper, the outside liquid may be filtered through filter paper, and the filtrate can be tested for ninhydrin reaction. Thus an entirely new method of blood serum diagnosis can be established.*

Hereat I performed the following test with a success, and I shall subsequently describe practical examples.

Materials Necessary for Kiutsi's New dialyzing Process.

1. Ninserin.
2. Dialyzing tube.
3. 1 % ninhydrin solution.
   Kept in a dark bottle.
4. Small cup.
   Capacity about 20.0 cc.

5. Large test tube.
   Convinient for boiling.
6. Test tube holder.
   Convinient for boiling.
7. 0.2 cc. capillary pipette.
   For measuring ninhydrin solution.
8. 1.0 cc. pipette.
   For measuring blood serum.
9. 10.0 cc. measuring cylinder.
   For measuring distilled water.
10. Distilled water.
11. Bunsen burner.
12. Funnel.
13. Filter paper.

Above 13 are all that are needed.

The dialyzing tube should first be tested whether it has the desired property, and its number must be at least double the number of blood serum samples to be tested. For instance, if five samples of blood serum are to be tested, there must be at least ten tubes. Each pair must have the same capacity, for when duplicate tests are made and the both reaction coincide it is very easy to decide the result; and in case any one must be discarded on account of any accident one is still left for interpretation.

Practical Application of Kiutsi's New Dialyzing Process.

I shall now explain this method taking an example in pregnance diagnosis.

Into a small cup 10 cc. distilled water is first put in, and 0.05 gram of Ninserin is added. Into a dialyzing tube 1.0 cc. of pregnant blood serum is put in, and this is placed into the above cup.

This is left at a room temperature to dialyze for 8 to 10 hours, and the liquid in the cup is filtered through filter paper into clean test tube. To this 0.2 cc. of ninhydrin solution is added and shaken a little. This mixture is then boiled for one minute and left still for 15 minutes. A brilliant purple color appears. It is positive. In case no color appears the reaction is said to be negative.

The reason why I allowed 8 to 10 hours for dialysis is to get the maximum reaction. But, any time from 4 to 18 hours the diagnostic reaction is distinct.

Experimental Steps.

The experiments with Kiutsi's new dialyzing method are conducted in three steps, namely the preliminary, the main, and the control.

The Preliminary Experiment.

On this experiment the change of Ninserin with distilled water toward ninhydrin solution was determined.

No.	Ninserin	Distilled water	Reactions.
1	0.005 gram	10.0 cc.	Negative
2	0.02 ,,	10.0 ,,	,,
3	0.03 ,,	10.0 ,,	,,
4	0.04 ,,	10.0 ,,	,,
5	0.05 ,,	10.0 ,,	,,
6	0.10 ,,	10.0 ,,	,,

From the above data, it can be seen that the Ninserin does not make any change when treated with distilled water for 10 hours as tested by ninhydrin solution. In other words, by treating so, no peptone or amino acid is formed.

The Main Experiment.

On this experiment whether or not Ninserin be resolved by pregnant's blood serum was determined.

No.	Ninserin	Materials, 1 grm. each.	Reactions.
1	0.05 gram	Pregnant blood serum	Positive
2	0.05 ,,	Distilled water	Negative
3	——	Pregnant blood serum	Negative

From the above it can be seen that Ninserin is resolved by pregnant's blood serum into peptone and amino acid.

The Control Experiment.

On this experiment whether or not my Ninserin be resolved by blood serum of other than pregnant was determined.

No.	Ninserin	Blood serum	Reactions
1	0.05 gram	Muscle tumor	Negative
2	0.05 ,,	Ovary cyst	,,
3	0.05 ,,	Navel	,,
4	0.05 ,,	Man	,,

The above data show that the Ninserin can not be resolved by any blood serum other than that of pregnant.

From the result thus far described, I am confident in saying that a perfect diagnosis of pregnance can be made with my new dialyzing method.

These results are what I expected from the begining. But as the results are so marked and distinct I can not help but feel happy.

Those who studied my new dialyzing method before can easily try the new dialyzing method and notice the markedness of the result. Because my improved dialyzing method is, as the readers may already know, to put dried substratum in the tube, but in new dialyzing method the dried substratum is placed *outside of the tube*, the latter method being the result of the application of the fact that the ferment has a property of passing through animal membrane freely.

By the discovery of the new method I directly found the following three distinct benefits.

1st. As the dialyzing tube is almost intact, it can be used comparatively long.

2nd. It is easy to clean after its use.

3rd. As it allows chemotaxis to act perfectly, the reaction is very marked. For, as a protective ferment has power of acting vigorously on its substratum, its property of chemotaxis is very sharp. Therefore, if the substratum, is on the outside of tube the ferment in inside of the tube will storm to it in a mass and passes out. Consequently in outer liquid comparatively more resolution of protein takes place than in the improved method in which the substratum is in the tube, and it is clear that the reaction also proportionately differ.

Thus by the discovery of the new method not only practical benefits are attained, but also the confirmation of my assertion of passing out of ferment into dialyzate is apparent.

Though by the foregoing studies I proved the presence of protective ferment in urin as well as in blood serum, there was a room still left for further study.

It is to determine whether the pregnant ferment isolated from urin is the same with special pregnant ferment which has been studied in the blood. To answer this question the following experiment was conducted.

Toward this object I performed the foregoing Kiutsi's new dialyzing process with pregnant ferment. Because, if this result be positive I can at once decide the following two cases.

1st. The protective ferment isolated from urin is the same thing with the protective ferment of blood.

2nd. The protective ferment isolated from urin passes through animal membrane as that of blood.

To test for the above, I put 2 ear-pickfuls of pregnant ferment into a dialyzing tube and added 1 cc. of distilled water. Then this tube was placed in a small cup containing 10 cc. of distilled water and 0.05 gram of Ninserin. After 4 hours the ninhydrin reaction was positive while all control tests were negative.

As said before, the pregnant ferment isolated from urin is entirely the same thing with that of blood serum. In other words, *the blood serum contains this pregnaut ferment and at the same time a considerable amount of protein and a small amount of protein splitting products which are not necessary for my purpose, while urin contains this pregnant ferment and at the same time a small amount of protein and a large amount of protein splitting products.*

I hope that the readers can understand thoroughly that this is the way that it becomes dialyzing method and filtration method.

At this end I shall like to add a few words in regard to the reaction of ninhydrin and Bieuret tests.

There are many who know these words, yet only a limited number of them know the reality of these tests. Hence these words which come from my experimental facts.

In Bieuret test, sodium hydroxide solution is usually added to the liquid to be tested. To this a dilute solution of copper sulphate is dropped in, and the appearance of purple color is detected. This test in satisfactory when plenty of the reacting substance is present and the tester is very keen observer of color. But, when test is very delicate, i. e., the reacting substance is very small in its amount, some other method must be pursued.

For the reason above, as I reported often before, it is necessary to employ layer test. In this method 33 % sodium hydroxide solution is added to the liquid to be tested in proportion of one of the former to three of the liquid to be tested. To this mixture 0.2 % copper sulphate solution is gradually added to form layers of the two liquids. Holding this straight and taking this near to white wall or against white paper the reaction is examined. Where two layers meet a purple ring can be noticed. Unless one is color blind one can by tnis method easily test the presence of a very delicate amount of peptone.

It is often met to hear uncertainty of ninhydrin test. This is no doubt due to the use of ninhydrin solution that has already lost its efficiency. Because, if the ninhydrin solution is exposed to snn light it looses its activity. Though it keeps longer when it is kept in a dark bottle, it is often met that it looses its activity in about one month. If one without realizing these facts uses any ninhydrin solution that has already lost its activity or that has been weakened the reaction may be negative. One who uses this solution must watch these points. Otherwise he may make a great mistake in interpretation of his test.

To test the efficiency of the ninhydrin solution is to boil it with protein, peptone, or amino acids. By this test the efficiency of the solution can easily be tested. But even such a simple test is considered irksome and very likely neglected. Therefore, I shall propose still more simple method of testing the efficiency of ninhydrin solution. *It is to watch the change of color of finger tip by moistening the finger tip with ninhydrin solution. If the solution is still active the finger tip turns purple in a short time.* This is the most simple and takes shortest time for the test.

Another thing to be pointed out as to the ninhydrin is the time of boiling. Abderhalden boil for one minute after appearance of first bubble, but I consider it best to boil for one minute after the liquid began to boil properly. When my method of boiling is employed the liquid usually diminishes to one third of its original volume. Therefore, if no watch is available it is safe *to boil so long as the liquid becomes 1/3 its original volume.*

The water solution of ninhydrin do not change its color to purple no matter how long it may be boiled, except it becomes golden yellow at the end. Therefore, over boiling does not do any harm to the ninhydrin reaction. *When the reaction is very weak, it may be a good idea to continue boiling, in the last one Cubic-centimeter of the liquid.*

The ninhydrin is sold usually in 0.1 gram quantity. Therefore, unless one uses it often, it is a good idea not to make it to a solution at once, but to divide it into two or three portions and make its 1 % solution as needed.

The ninhydrin does not dissolve in water at once. But if it is shaken for some time it dissolves quicker.

By all sudden, my filtration method and new dialyzing method became successful. With practice observations were gained. The observations opened the way to hypothesis. To the hypothesis, the conclusions and generalizations are born, and these catch all the phenomena of the world exposing it realistically to the public. Ah, at last the protective ferment came into my hand and dried substratum is now sleeping on my palm! The cohesion of the both brings reactions before our eyes.

Thus Kiutsi's filtration process made a healthy progress and I thank the public for this success.

TWO HOURS!

This is the result that I got in performing the test.

And I shall add at this end that all what have been said in this pamphlet are perfectly applicable to the diagnosis of all animals.

(the end)

Oct. 1, 1914.

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QP 211.K62

Kiutsi's isolation of protective ferment

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